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BACTERIOCHLOROPHYLL *a* CATION RADICAL IN SOLUTION AND IN REACTION CENTERS OF *RHODOPSEUDOMONAS SPHAEROIDES* RESONANCE RAMAN SCATTERING

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Summary

Resonance Raman spectra of the π-cation of bacteriochlorophyll *a* in solution at 30 K are reported and discussed. Outer C$^{\cdots}$C bonds of the pyrroles and the methine bridges are weakened by the ionization, while C$^{\cdots}$N and Mg-N bonds remain essentially unaffected. Resonance Raman spectra of reaction centers suggest that the positive charge on *P*-870^{+} should be localized on a single bacteriochlorophyll molecule by the lifetime of the scattering process ($\approx 10^{-13}$ s).

Bacteriochlorophyll (BChl) has been identified as the primary electron donor in reaction centers of photosynthetic bacteria. The light-induced, one electron oxidation of BChl produces a free radical which bears close similarity with the in vitro BChl^{+} π-cation, as shown by ESR, ENDOR and electronic absorption spectra. It has been concluded, however, from ESR and ENDOR data, that in the reaction center the unpaired electron of the free radical delocalizes over two BChl molecules, by the time scales of these techniques [1].

We obtained resonance Raman spectra of the cation radical of BChl *a* in vitro at low temperature and investigated *P*-870^{+} using this technique, whose intrinsic time scale is much shorter than that of ESR and ENDOR.

BChl *a* was extracted from *Rhodopseudomonas sphaeroides* and purified as previously described [2]. The samples were dried as in Ref. 3 and handled in a dry nitrogen atmosphere. The cation was produced by revers-

Abbreviations: BChl, bacteriochlorophyll; Chl, chlorophyll.

ible formation of a 1:1 charge-transfer complex with molecular iodine in methanol or dichloromethane solution [4].

Resonance Raman spectra of 10^{-3} M BChl a^+ in methanol at 30 K were obtained with 441.6 nm (He-Cd laser), and 514.5 and 528.7 nm (Ar laser) excitations, using spectroscopic methods described previously [2,5]. More than 40 bands were observed in the 100—1700 cm^{-1} region (Table I and Fig. 1). Spectra obtained at the same concentration in CH_2Cl_2 were nearly identical to those in CH_3OH but contained Raman bands of the solvent.

Cooling by itself has no noticeable effect on intensities or frequencies of resonance Raman bands of chlorophyll and of BChl [2,5] and no greater effect is expected for $BChl^+$. Indeed, the two resonance Raman bands previously reported for $BChl^+$ at room temperature at 1598 and 1578 cm^{-1} [6] correlate within experimental uncertainty with the major 1590 cm^{-1} band and the 1570 cm^{-1} shoulder of the present spectra, respectively.

Variations in intensities and frequencies of resonance Raman bands of BChl follow the changes in π-electron distribution on the conjugated part of the molecule which are induced by the ionization. Resonance Raman spectra of $BChl^+$ obtained by resonance on the 520 nm band, i.e. those

TABLE I

FREQUENCIES (cm^{-1}) OF RESONANCE RAMAN BANDS OBSERVED FOR BACTERIOCHLOROPHYLL a^+, CHEMICALLY PRODUCED IN METHANOL OR DICHLOROMETHANE, 30 K, EXCITATION RANGE 441.6—528.7 nm

BChl a^+	Shift with resp. to BChl a	Involvement of $\nu(C^{\cdots}N)$ and $\delta(C^{\cdots}N)$ [c]	BChl a^+	Shift with resp. to BChl a
1625 vw sh [a]	—5	—	905 w	+3
1590 s	—20	—	860 vw	0
1570 wsh	—7	—	?845 vw	
?1560 w(S) [b]		—	830 w	
1545 vw sh	0	—	802 m	+6
1508 w	—7?	+	765 w	—3
1485 w	—10?	—	740 w	+3
1463 ew			720 vw	+6
1432 ew, w(S)	—15	+	690 vw, vw sh(S)	0?
1412 w	—10	+	673 w	—5?
1375 w	—5	+	612 vw, w(S)	—13
1350 w sh, w(S)	—11	+	585 vw	—2
1325 s, vw sh(S)	—20	+	490 ew, b	—2
1290 w	0	++	445 vw, w(S)	—3
1250 w	0	+	405 w, vw(S)	0
1215 vw, b	0	++	385 vw	—5
1190 vw sh, vw(S)	0	+	355 vw	0
1170 w	+5	++	295 vw	0
1152 vw sh	+6	+++	?260 ew	—5
1120 vw, b	0	+++	200 ew [d]	0
1070 ew, w(S)	0	++	110 vw (S)	
1035 w (S)	0	+		
1007 vw sh	—1	+		
975 ew	+3	(++)		
933 m, w(S)	0	++		

[a] Overall uncertainty on frequencies ± 5 cm^{-1}. Relative intensities at 514.5 nm and at 441.6 nm (S); s, strong; m, medium; w, weak; v, very; e, extremely; sh, shoulder; ?, doubtful.

[b] Phorbin band ?

[c] From the average frequency shift on $^{14}N \rightarrow {}^{15}N$ substitution in both Chl a and Chl b [11].
—, $\Delta\nu/\nu \leqslant 10^{-3}$; +, $10^{-3} < \Delta\nu/\nu \leqslant 4 \cdot 10^{-3}$; ++, $5 \cdot 10^{-3} \leqslant \Delta\nu/\nu \leqslant 9 \cdot 10^{-3}$; +++, $9 \cdot 10^{-3} < \Delta\nu/\nu$.

[d] This band might in part arise from complexed I_2.

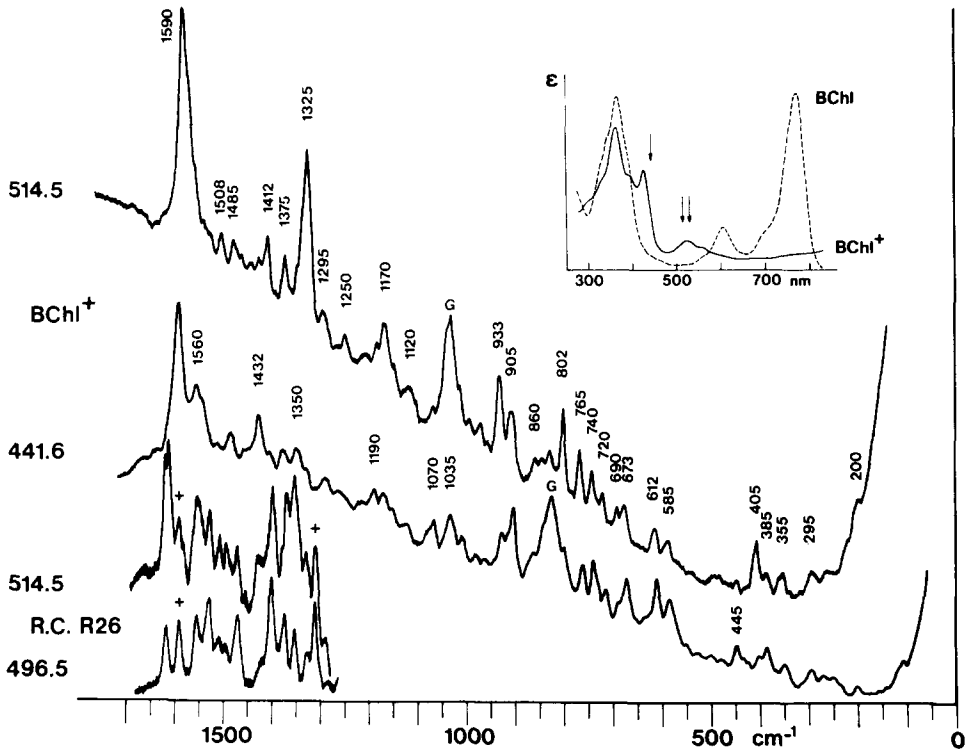

Fig.1. Resonance Raman spectra of 10^{-3} M BChl a^+ in methanol, 30 K, resolution at 1000 cm^{-1}, 7 cm^{-1} (514.5 nm), 10 cm^{-1} (441.6 nm); G, spurious lines. Bottom: Resonance Raman spectra (partial) of reaction centers from *Rps. sphaeroides*, R26 strain, 30 K, resolution at 1500 cm^{-1}, 7 cm^{-1}. Crosses indicate the two lines tentatively attributed to *P*-870$^+$. Inset: Electronic absorption spectra of BChl a^+, 1:1 charge-transfer complex with iodine in methanol, and of BChl *a*. Arrows indicate the excitation wavelengths used: 441.6, 514.5 and 528.7 nm.

excited at 514.5 and 528.7 nm, retain intensity distribution close to that observed for BChl in Q_x resonance [2]. The main discrepancy occurs at 1545 cm^{-1}, a band strong for BChl and weak for BChl$^+$. Resonance Raman spectra of BChl$^+$ excited at 441.6 nm, i.e. with resonance primarily on the 423 nm band (likely the B_x transition of BChl$^+$ [7]) differ from the preceding by a weakened 1327 cm^{-1} band, just as the resonance Raman spectra of BChl obtained in Soret resonance at 363.8 nm differ from those obtained in Q_x resonance [2]. At this wavelength, however, BChl$^+$ does not exhibit any enhancement of a band at 1290 cm^{-1}, contrary to BChl excited at 363.8 nm, at the top of the B_y transition [2]. This indicates that the 1290 cm^{-1} mode should be exclusively coupled to *y*-polarized transitions. These observations on relative intensities indicate that the conformation and molecular symmetry of the conjugated part of BChl remain essentially unaltered on removal of one π-electron, and that the 520 nm electronic band of BChl$^+$ most likely corresponds to its Q_x transition.

The second row of Table I contains the average frequency shifts observed on the bond-stretching frequencies (900—1700 cm^{-1}) of BChl upon ionization, taking into account both Q_x and Soret resonance data. All of these shifts are less than 20 cm^{-1}, indicating that the ionization does

not much alter the bonding of the dihydrophorbin ring. ENDOR data indeed showed that the unpaired electron had a significant probability of presence on the non-conjugated carbons of pyrroles II and IV [8]. Nevertheless, about one-half of the bond-stretching frequencies decreases upon ionization, as a result of a weakening of the corresponding bonds when a bonding π-electron is lost by BChl. Most of the downshifted bands occur in the higher frequencies region (1300—1700 cm^{-1}). They correlated with resonance Raman bands of Chl *a* and/or of Chl *b* which presented no or very low sensitivity to isotopic substitution of the pyrrolic nitrogens [9], i.e., they most probably arise from modes with low participation of $\nu(C\cdots N)$ and of $\delta(C\cdots N)$ coordinates (third row of Table I). On the other hand, $BChl^+$ bands likely to involve high nitrogen participation do not shift, or even shift to higher frequencies. Low frequency modes likely to involve motions of the magnesium atom, at 355, 295 and 200 cm^{-1} [2,5,9] are insensitive to the ionization. This latter point is quite consistent with a recent observation by Druyan et al. [10] that Chl a^+ does not bear any detectable spin density on its Mg atom.

Hence, the formation of the $BChl^+$ cation primarily results, in the conjugated part of the dihydrophorbin ring, in a weakening of $C\cdots C$ bonds, including the methine $C_a\cdots C_m$ bridges, whose stretching coordinates should predominate in the 1590 cm^{-1} mode [2]. The $C_a\cdots N$ bonds should be affected very weakly, as well as the $Mg\text{-}N_4$ bonding. These conclusions appear to be consistent with the evaluations of unpaired spin densities derived from ESR and ENDOR spectra [8,10]. Raman data however suggest that the unpaired spin densities on the methine carbons (or those on the nitrogens) might have been underevaluated in Ref. 8.

Reaction centers from *Rps. sphaeroides*, strain R26 [11] treated with excess ferricyanide and excited at 496.5, 501.7 and 514.5 nm (Ar laser) at 30 K yielded very weak resonance Raman spectra superimposed on strong backgrounds from spurious fluorescence (Fig. 1) which forbade any observation in the 440—490 nm range. Most of the observed bands arise from bacteriopheophytin, some of them from BChl. Two bands at 1590 and 1315 cm^{-1} have no homologues in the resonance Raman spectra of reaction centers excited in the Q_x bands of BChl and of bacteriopheophytin [2]. Reaction centers excited in the Soret band at 363.8 nm yield a strong resonance Raman band at 1585 cm^{-1} arising from bacteriopheophytin (Lutz, M., unpublished result). However, isolated bacteriopheophytin excited at 514.5 nm does not yield this resonance Raman band. No other constituent of the reaction center appears likely to yield any of these two bands. Hence, we tentatively attribute them to $P\text{-}870^+$. All our attempts to observe resonance Raman spectra of chemically reduced reaction centers in this spectral region failed, however, due to a strong increase of the spurious fluorescences which resulted from the treatment. Untreated centers were kept in their oxidized state by the laser beam and yielded the same spectra as the ferricyanide-treated centers.

The frequencies of the two resonance Raman bands at 1590 and 1315 cm^{-1} are downshifted from those of isolated BChl by the same and higher values than those of the $BChl^+$ monomer in methanol respectively.

Hence, the π-bond orders of the BChl C$\cdots$C bonds involved in the ionization of *P*-870 should decrease by at least the same amounts as for monomeric BChl a^+. This indicates that the unpaired electron, which appears delocalized over two BChl of the reaction center by ESR and ENDOR time scale, should be localized on a single BChl molecule by the shorter time scale of the resonance Raman effect on BChl (about 10^{-13} s).

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